PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4 -32701A/USN	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/10675	International filing date *(day/month/year)* 25.09.2003	Priority date *(day/month/year)* 26.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant NOVARTIS AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.04.2004	24.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hermann, P Telephone No. +49 89 2399-7109

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10675

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-16 as originally filed

Sequence listings part of the description, Pages

1-22 as originally filed

Claims, Numbers

1-9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-9 (all partially)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-9 (all partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-9 (partially) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Subject-matter for which no international search report has been established have not been examined (cf Rule 66.1 PCT).

Therefore, claims 1-9 have been examined with respect to the provisions of Art.33(1) PCT (i.e. novelty, inventive step and industrial applicability), only insofar as they relate to the subject-matters of invention 1, i.e. a PCR based method for detecting mutations responsible for amino acid changes G463E or G463V in the B-RAF gene, comprising a first detection primer SEQ. ID. nos 1, 15 and 29 and a second primer SEQ. ID. no 43; or a first detection primer SEQ. ID. nos 2, 16 and 30, and a second primer SEQ. ID. no 44.

Re Item IV

Lack of unity

1. Reference is made to the following documents:

   A: GB-A-2327497
   B: W0-A-0047766
   C: Davies H. *et al.* - 'Mutations of the BRAF gene in human cancer' - 2002 - *Nature*, **417**: 949-954
   D: Rajagopalan H. *et al.* - 'RAF/RAS oncogenes and mismatch-repair status' - 2002 - *Nature*, **418**: 934

2. This International Examination Authority is in agreement with the International Search Authority and considers that there are 8 inventions covered by the claims indicated as follows:

   I. Claims: 1-9 partially
   PCR-based method for detecting mutations responsible for amino acid changes G463E or G463V in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 1,15 and 29 and a second primer SEQ ID No 43; or a first detection primer

from SEQ ID Nos 2,16 and 30, and a second primer SEQ ID No 44.

II. Claims: 1-9 partially
PCR-based method for detecting mutations responsible for amino acid changes G465A or G465E or G465V in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 3, 17 and 31 and a second primer SEQ ID No 45; or a first detection primer from SEQ ID Nos 4, 18 and 32, and a second primer SQ ID No 46; or a first detection primer from SEQ ID Nos 5, 19 and 33; and a second primer SEQ ID No 47.

III. Claims: 1-9 partially
PCR-based method for detecting mutations responsible for the amino acid changes G468A or G468E in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 6, 20 and 34 and a second primer SEQ ID No 48; or a first detection primer from SEQ ID Nos 7, 21 and 35, and a second primer SQ ID No 49.

IV. Claims: 1-9 partially
PCR-based method for detecting G1753A mutation responsible for the amino acid change E585K in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 8, 22 and 36, and a second primer SEQ ID No 50.

V. Claims: 1-9 partially
PCR-based method for detecting T1782G mutation responsible for the amino acid change F594L in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 9, 23 and 37, and a second primer SEQ ID No 51.

VI. Claims: 1-9 partially
PCR-based method for detecting G1783C mutation responsible for the amino acid change G595R in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 10, 24 and 38, and a second primer SEQ ID No 52.

VII. Claims: 1-9 partially
PCR-based method for detecting B-RAF mutations responsible for amino acid changes L596V or L596R in the B-RAF gene, comprising a first detection primer from SEQ ID Nos 11, 25 and 39, and a second primer SEQ ID No 53; or a first

detection primer from SEQ ID Nos 12, 26 and 40, and a second primer SEQ ID No 54.

VIII. Claims: 1-9 partially
PCR-base method for detecting mutations responsible for amino acid changes V599E or V599D in the B-RAF gene wherein for the V599E change is selected from either a first set comprising a first detection probe from SEQ ID Nos 13, 27 and 41, and a second primer SEQ ID No 55; or a second set comprising a first detection primer from SEQ ID Nos 57, 59, 60, 61 and 62, and a second primer SEQ ID No 58, or for the V599D change is selected from a first detection primer from SEQ ID Nos 14, 28 and 42, and a second primer SEQ ID No 56.

The present application discloses oligonucleotide primers for use in a method for the detection of mutations in the human B-RAF gene. Two types of primers are disclosed for use in an allele specific PCR based detection method: detection primers (allele specific primers), wherein the 3' end of each detection primer is complementary to a specific mutated base on a first DNA strand of the B-RAF gene and, second primers - wherein each second specific primer is used with a specific first detection primer - each complementary to the opposite DNA strand of the B-RAF gene. The polymerase used in the method is one devoid of 3'- 5' exonuclease activity, and the detection primer is so selected that a detectable amplification product is produced only when the mutation is present.

The common concept linking inventions 1-8 can be seen as the provision of ARMS (Amplification Refractory Mutation System) -type primers for detecting the presence of mutations in the human B-RAF gene for diagnostic purposes.

However, the claimed method, i.e. ARMS is already commonly practised in the prior art (cf. the whole disclosure of **A** and **B**) as a PCR based method suitable for detecting mutations in genes. Thus the detection method claimed in the present application is not novel, and the selection from known sequences of primers suitable to be used in such technique is considered to be common practise for the skilled person.

Moreover, B-RAF gene mutations associated with human cancers are already documented in the prior art on the basis of nucleic acid sequence analysis and reported

in **C** and **D**. Indeed, many of the B-RAF mutations shown in Tables 1, 2, 3 and 5 have previously been disclosed in these citations (cf. **C** tables 2 and 3 and **D** Table 1) . Thus the disclosure of B-RAF mutations is per se not novel, likewise the association of B-RAF mutations with human cancer is also not novel.

In view of the above prior art, the 8 different problems underlying the present application can be seen as the provision of ARMS-type primers for detecting the presence of mutations in the human B-RAF gene for diagnostic purposes. The respective problems are solved by inventions 1-8 each providing a specific oligonucleotide pair, each comprising a detection and a second primer to each mutational locus.

Therefore, due to the structural differences of the primers, and the absence of any further special technical features which could be identified as "special technical feature" bringing a new and inventive contribution over the prior art, or representing a common inventive link between the subject-matters of inventions 1-8 in the sense of Rule 13.2 PCT, the present application does not meet the requirements of 13.1 PCT with respect to unity of invention, and is considered to consist of the 8 separate inventions given above.

3. An international search report has been done with respect to inventions 1 and 8. . However, the applicant has chosen not to pay the additional fee and at the same time did not restrict the claims to one of the aforementioned inventions; the examination has therefore been carried out on the subject-matter relating to invention 1.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: Davies H *et al.* - "Mutations of the BRAF gene in human cancer" - 2002 - *Nature*, **417**: 949-54

D2: Rajagopalan H *et al.* - "RAF/RAS oncogenes and mismatch-repair status" - 2002 - *Nature*, **418**: 934

D3: WO-A-00/47766 (Gibson Neil James; Foy Carole Ann (GB); Astrazeneca UK Ltd (GB); H) 17 August 2000

D4: WO-A-93/04186 (US Government) 4 March 1993

D5: Sithanandam G *et al.* - "Complete coding sequence of a human B-RAF cDNA and detection of B-RAF protein kinase with isozyme specific antibodies" - 1990 - *Oncogene*, **5**: 1775-1780

2. Novelty (Article 33(2) PCT)

2.1 None of the document cited in the International Search Report discloses the method of present claims 1 or 2, or the primers of claims 3-9. Claims 1-9 thus fulfill the requirements of Article 33(2) PCT for novelty of their respective subject-matters.

3. Inventive step (Article 33(3) PCT)

3.1 Document D1 and D2 which are both disclosing a similar method for the detection of the mutation G463V or G463E in the gene encoding BRAF can be both independently considered closest prior-art for the method of present claim 1. The methods of D1 or D2 comprise amplifying the portion of the gene containing said mutation by PCR, and sequencing said amplified portion in order to identify the codon encoding the amino acid in position 463. The method of present claim 1 differ from the method of D1 or D2 by the fact that the codon encoding amino acid in position 463 is determined using a multiplex Amplification Refractory Mutation System (mutiplex ARMS) which is in fact a simplified and quicker method for mutation detection.

In view of the prior art at hand, the problem to be solved by the method of claim 1 can be seen in the provision of an improved method for the detection of mutation in BRAF encoding nucleotidic sequence.

The solution to said problem, i.e. the use of multiplex ARMS together with primers specific for the detection of a potential mutation in a specific codon, is however not considered inventive because at the relevant filing date multiplex ARMS together with its advantages/performances was already well known in the art, as shown in D3 (cf. D3 p. 1 line 1 - p. 23 line 32, claims) and the choice of primers to use in multiplex ARMS for the detection of known mutation in gene for which the complete sequence is known (see

D4 and D5) represent an obvious design procedure the skilled person would perform without exercise of inventive skills. Therefore the method of present claim does not involve an inventive step and thus do not meet the requirements of Article 33(3) PCT.

3.2 For the same reasons as expressed above under point 3.1, the method of claim 2 is not considered to involve an inventive step.

3.3 Since the mutation expected to be detected was already known in the art (see point 3.1 above) the oligonucleotide primers contained in claims 3-9 do not appear to bring any unforeseeable effect. Said oligonucleotide primers are therefore not considered inventive and claims 3-9 lack inventive step and do not meet the requirements of Article 33(3) PCT.